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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before The Board of Patent Appeals and Interferences

In re PATENT APPLICATION of

Gregory P. WINTER et al Atty. Ref: **620-3**

Serial No. **07/796,805** Group: **1805**

Filed: **November 25, 1991** Examiner: **J. Ketter**

For: **SINGLE DOMAIN LIGANDS, RECEPTORS COMPRISING SAID LIGANDS, METHODS FOR THEIR PRODUCTION AND USE OF SAID LIGANDS AND RECEPTORS**

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October 26, 1993

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

APPEAL BRIEF

Sir:

This is an appeal from the decision of the Examiner finally rejecting claims 33-63. As will become evident from the following discussion, the Examiner's prior art rejections are in error and, as such, reversal of the same is solicited.

1. STATUS OF THE CLAIMS

A. The following Claims are presently pending in this application:

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33-63.

The following Claims have been cancelled during prosecution to date.

1-32.

2. STATUS OF AMENDMENTS

The Amendment after final rejection filed July 20, 1993 included no claim amendments.

3. SUMMARY OF INVENTION

The subject invention relates to a method of cloning sequences (target sequences) each containing a sequence encoding at least part of an immunoglobulin variable domain, which method comprises

providing *a sample repertoire of nucleic acid containing target sequences*, and

using forward and back primers in the copying and cloning of the target sequences for expression of *a repertoire of proteins each comprising at least part of an immunoglobulin variable domain*, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of the target sequences.

Essential to the present invention as claimed is a sample repertoire of nucleic acid containing target sequences. The sequences code for immunoglobulin variable domains. By definition, such domains are variable in sequence. The present inventors were the first to recognize that, contrary to all expectations, the ends of the variable domain genes are

sufficiently conserved to allow one to make primers which can be used in cloning an entire repertoire of variable domain genes.

The term "repertoire" has a special significance in relation to antibodies/immunoglobulins and their encoding genes. For instance, the "antibody repertoire" of a mouse is the estimated between 10^6 and 10^9 different molecules which the animal is able to make. The term "repertoire" connotes the number of different molecules being apparently large enough to ensure that there will be an antigen-binding site to fit almost any antigenic determinant. Certainly the term conveys the idea of diversity among a large population.

The diversity of the genes encoding the repertoire of antibodies is generated in a number of ways, including mutation and gene rearrangement. Significant in the present context is the indisputable fact that the repertoire of variable domain genes, though not as diverse as the final expressed protein repertoire, is highly diverse, each individual having quite a large number of genes encoding variable domains, each sequence being different. (The term "variable domain" is used because the sequences are inherently variable, unlike the antibody "constant domain", which is conserved.) This is illustrated by experimental data in the subject application. Attention is drawn to page 42, Example 2. There it is described how, using the present invention, 48 V_H gene clones were obtained and sequenced. The sequencing revealed that each of the 48 clones was unique: each clone was different.

The power of the present invention to allow the cloning for expression of a larger repertoire of antibody variable domains is in stark contrast with the situation which existed in the prior art. As has been explained previously, and is discussed on page 22 of the

specification, prior to the subject invention, cloning of even a single immunoglobulin variable domain coding sequence required many steps. Individual probes were used in the isolation of a particular sequence of interest. This required sequencing of the N-terminal region of an antibody to enable design of specific probes which would be used to isolate a particular coding sequence, and then perhaps amplify it. The cloning of each gene sought after would involve all these steps.

Evidently, the cloning of a vast repertoire of immunoglobulin variable domain coding sequences was beyond the comprehension of one skilled in the art. With so many different sequences in the repertoire it was inconceivable that a repertoire could be cloned for expression. The sheer amount of work involved would have been phenomenal.

The present inventors for the first time conceived of the idea of the feasibility of cloning for expression a repertoire of immunoglobulin variable domain sequences AND for the first time provided one skilled in the art with the know-how required to put the idea into practice.

4. THE REFERENCES

The following references have been cited and applied to finally reject all pending claims herein:

Patent No.	Country	Patentee	Date
3,683,195	US	Mullis et al	7/87
4,983,728	US	Herzog et al	7/88
4,978,743	US	Schoemaker et al	12/90

Author	Citation	Date
Skerra et al.	Science, 240, 1038-1041	5/88
Kabat et al.	U.S. Dept. HHS, p 494-525	1987

5. THE REJECTIONS OF RECORD

1. Claims 33-63 have been finally rejected under 35 U.S.C. 103 as allegedly being unpatentable over Mullis et al.

2. Claims 38-45 and 57-63 have been finally rejected under 35 U.S.C. 103 as allegedly being unpatentable over Skerra et al in view of either Mullis et al or Herzog et al, and in view of Kabat et al.

3. Claims 34-37 and 46-56 have been finally rejected under 35 U.S.C. 103 as allegedly being unpatentable over Skerra et al in view of either Mullis et al or Herzog et al and in view of Kabat et al, as applied to claims 38-45 and 57-63 above, and further in view of Schoemaker et al.

6. ISSUES

The following issues are presented for purposes of this appeal.

1. Whether all claims are unpatentable under 35 U.S.C. §103 by virtue of Mullis et al.

It is the position of the Examiner that the "claims employ the methods disclosed by Mullis et al merely using different starting materials. . .", that "novelty in the starting materials and/or final product does not lend patentability to an art-known process of making"

and that "it would have been obvious to one of ordinary skilled in the art to have employed the PCR methods taught by Mullis et al to amplify any known target DNA molecule." "The motivation to have done so would have been that PCR is and was known to be a generally applicable technique to a broad range of DNA molecules, as is indicated in, e.g., Mullis et al at column 2, fourth full paragraph."

2. Whether Claims 38-45 and 57-63 are unpatentable under 35 U.S.C. §103 by virtue of Skerra et al in view of either Mullis et al or Herzog et al, and in view of Kabat et al.

It is the position of the Examiner that "it would have been obvious to one of ordinary skilled in the art to have modified the teachings of Skerra et al [the cloning and expression in *E. coli* of functional portions of immunoglobulin gene] by using mixed primer PCR, as taught by either Mullis et al [the use of a mixture of primers for ambiguous target sequences (column 8, first full paragraph)] or Herzog et al [the use of a mixture of primers to amplify related but different DNA sequences], based upon the DNA sequences taught by Kabat et al [cited to demonstrate that a large compilation of constant domain DNA and protein sequences of immunoglobulins, flanking the variable domains, was known in the art].

3. Whether Claims 34-37 and 46-56 are unpatentable under 35 U.S.C. §103 by virtue of Skerra et al in view of either Mullis et al or Herzog et al, and in view of Kabat et al, as applied to claims 38-45 and 57-63, and further in view of Schoemaker et al.

It is the position of the Examiner that "it would have been obvious to one of ordinary skill in the art to have practiced the invention of claims [38-45 and 57-63] with the modification of expressing the Ig fragments together as heterochain antibodies"; "The

motivation to have done so would have come from Schoemaker et al, column 2, fourth full paragraph, where it is disclosed that heterochain antibodies can be superior to the 'parental' antibodies from which the chains were derived."

7. GROUPING OF CLAIMS

To simplify the issues presented in this appeal, all of the Applicant's claims, 33-63, may be grouped, and thus considered as standing or falling together.

8. ARGUMENTS

The Examiner has provided no evidence that one of ordinary skill in the art had even thought of providing an expression repertoire of variable domain sequences.

Prior Art Rejection 1

Mullis et al

In rejecting all claims as unpatentable over Mullis et al (see rejection 1, above) the Examiner ignores the fact that those skilled in the art would not have believed that the process of Mullis et al could be applied in the context of cloning of an immunoglobulin variable domain repertoire. This being so, the obviousness rejection ("novelty in the starting materials and/or in the final product does not lend patentability to an art-known process") should be reversed.

In fact, the process as taught by Mullis et al is not conceivably employable in the cloning for expression of a repertoire of V domains. The Examiner contends that "PCR is and was known to be a generally applicable technique to a broad range of DNA molecules."

There is absolutely nothing in Mullis et al to suggest that one could clone a repertoire of V domains using the technique or a variation on it. The most that can be said is that the technique as disclosed by Mullis et al could perhaps have been used to clone *individual* immunoglobulin genes, by isolating an antibody, sequencing it, designing specific primers, taking into account the degeneracy of the genetic code (which may require the use of a pool of primers to ensure that one will match the underlying DNA sequence, given the fact that for any given amino acid sequence there will be a number of possible encoding nucleotide sequences), making the primers and probing for and amplifying the sought-after DNA. This process would have had to have been performed for *each and every variable domain gene* in the repertoire, a task which would have been inconceivable in view of the enormity of the genetic diversity within the repertoire.

The position that "Mullis et al indicate that their technique can be used to clone any sequence using 'suitable' primers, *therefore* the present invention is obvious" is logically insupportable. For a repertoire of inherently variable V domain sequences, the possibility of the existence of suitable primers was not considered. The approach of Mullis et al to sequence the end of *each and every sequence* to be cloned and then design and make primers specific for each one would not be feasible for a repertoire of millions of sequences, as discussed.

The present inventors were first to recognize that, *contrary to all expectations, the ends of the V genes are sufficiently conserved to allow one to make primers which can be used in cloning an* ***entire*** ***repertoire*** *of variable domain genes*. There is absolutely no suggestion of this in the prior art.

Regarding use of "mixed primers" it should be noted that the only mention of these by Mullis et al is in the context of providing a pool of primers containing sequences representing all possible codon variation based on degeneracy of the genetic code. The rationale is that, where the correct nucleic acid sequence is unknown, because the amino acid sequence available could be encoded by a multitude of different nucleic acid sequences, a pool of primers is used representing all possible underlying sequences, in order to make sure that one of them is correct. This kind of approach is one avoided by the present invention. It is now not necessary to sequence immunoglobulins *individually* and contact a pool of primers for each one. Following PCR as taught by Mullis et al would never have lead one of ordinary skill in the art to conceiving of cloning a repertoire of immunoglobulin variable domain-encoding genes.

Prior Art Rejection 2

With regard to the second and third prior art rejections, there would have been no motivation for one of ordinary skill in the art to have combined the cited references in the manner suggested by the Examiner: the Examiner has employed hindsight reasoning. In fact, the Examiner's combination of documents does not lead to the present invention. The hindsight issue will be discussed in detail below after Mullis, Skerra, Herzog and Kabat are discussed.

In prior art rejection 2, the Examiner's stated position, taking into account the comments made in both Office Actions, is that "it would have been obvious to one of ordinary skilled in the art to have modified the teachings of Skerra et al [the cloning and expression in *E. coli* of functional portions of immunoglobulin gene] by using mixed primer

PCR, as taught by either Mullis et al [the use of a mixture of primers for ambiguous target sequences (column 8, first full paragraph)] or Herzog et al [the use of a mixture of primers to amplify related but different DNA sequences], based upon the DNA sequences taught by Kabat et al [cited to demonstrate that a large compilation of constant domain DNA and protein sequences of immunoglobulins, flanking the variable domain, was known in the art].

Indeed, Mullis et al in the first full paragraph of column 8 state "Any specific nucleic acid sequence can be produced by the present process." However, Mullis et al go on to state "It is only necessary that a sufficient number of bases at both ends of the sequence be known in sufficient detail so that two oligonucleotide primers can be prepared which will hybridize to different strands of the desired sequence . . . ." Mullis et al teach that some sequence information is required for *each* sequence to be amplified, in order for it to be possible to design and make the requisite primers.

Skerra et al

The Examiner's stated view of this document is that it teaches the cloning and expression in *E. coli* of DNA segments encoding Fv fragments and is therefore relevant to the claimed invention. What the document discloses is the synthesis of DNA encoding both a light chain variable domain and a heavy chain variable domain and the expression of this DNA in *E. coli* to produce a folded Fv fragment.

The document is wholly unconcerned with the cloning for expression of a repertoire of immunoglobulin variable domain sequences. It is therefore scarcely relevant to the claimed invention, providing no suggestion of the idea nor how to achieve it.

Skerra et al might demonstrate the cloning and expression of immunoglobulin variable domains of an antibody but it provides absolutely no clue on how to clone a repertoire of immunoglobulin variable domain sequences for expression as required by the present invention as claimed.

Herzog et al

Firstly, this document is unconcerned with immunoglobulin variable domains and repertories of these, being only specifically concerned with human papilloma virus nucleic acid. It provides no motivation to clone repertoires of immunoglobulin variable domain encoding sequences for expression and certainly no teaching as to how to do this. Its teaching, insofar as it can be considered to be relevant to anything other than human papilloma virus, is actually quite similar to that of Mullis et al.

Explicitly, in Example 6, Herzog et al describe that the sequences of the primers were "selected so as to amplify specifically the fragment of the gene of the virus to which they were addressed, with the exclusion of all the others" (emphasis added). For *each sequence of interest* (each HPV type) Herzog et al used a different primer. Like Mullis et al, the teaching is that a specific primer must be used for each sequence of interest.

As discussed, where a repertoire of immunoglobulin variable domains is being cloned for expression, it would be inconceivable to use a different primer for each sequence. Instead, as provided by the present invention, primers which bind to more than one sequence can be used, surprisingly given the inherently variable nature of the variable domains.

Of course, even if Herzog et al taught that one primer could be used to bind more than one sequence, which it does not, it would not point to the present invention as claimed

because there is no contemplation of immunoglobulin variable domains in the document and <u>nothing to lead one of ordinary skill in the art to recognize the unexpected sequence conservation within the variable domains</u>.

<u>Kabat et al</u>

The importance given to this document by the Examiner is out of all proportion to the effective technical teaching it provides. It is a raw compilation of sequence information on immunoglobulin genes, providing no suggestion of how such data might or should be used. <u>To look at the document now and say that it is obvious that there is sequence conservation such as to allow primers to be made and used in the cloning of immunoglobulin variable domain gene repertoires is to fall victim to hindsight</u>.

As discussed, there is no evidence that prior to the present invention anyone was even looking for such conservation. Without the <u>idea</u> of cloning a repertoire of V domain sequences (provided first by the present inventors) there was absolutely no motivation for anyone to look for sequences which would enable repertoire cloning.

Furthermore, it was far from obvious that the sequences required by the claims exist. By definition, immunoglobulin variable domains are <u>inherently variable</u>. The Examiner says that Kabat et al "was cited to demonstrate that a large compilation of constant domain DNA and protein sequences of immunoglobulins, flanking the variable domains, was known in the art." This is not at all relevant given the requirements of the claims for primers specific for a sequence at or adjacent the 3' end of both the sense and antisense strands of the target sequences, i.e., primers for <u>both</u> ends of the targets. It is particularly surprising that the 3' end of the antisense strand, corresponding to the 5' end of the sense strand, should be

conserved sufficiently to allow the practice of the present invention. The presence or absence of a constant domain says absolutely nothing about this end of an immunoglobulin variable domain.

One might draw a parallel with the pioneering work of Watson and Crick in elucidating the structure of DNA. The basic raw data needed for them to be able to determine the structure of DNA was available to them for some considerable time. They knew the size of the bases, that the amounts of cytosine and guanosine present are the same, and the amounts of adenine and thymidine present are the same, and they had lots of crystallographic data. Once they had arrived at the correct structure it was easy to see, with hindsight, that the answer was elegantly simple. However, the 20/20 vision of hindsight did not detract from the fact that their piecing together of the available information and the flash of inspiration required for success was sufficient for them to be awarded a Nobel prize.

Significantly, Watson and Crick were specifically looking for the answer and had motivation to combine all the pieces of information they had. In the present case, as discussed, there is no evidence that anyone had even conceived of the idea of cloning a repertoire of immunoglobulin variable domains for expression and were motivated to look for a way to put the idea into effect. Without the idea, there was no motivation to look. Even with the idea, the answer is not obvious.

The Examiner's Rejection is Based on Hindsight Reasoning

It is submitted that the Examiner has employed hindsight in constructing obviousness rejections using the present invention as a "blueprint" for combining references which do not themselves provide suggestion or motivation for such combination. It is further submitted

that the combinations of references which have been made by the Examiner do not anyway afford a legal conclusion of obviousness of the present invention as claimed.

It is well established that in order for an Examiner to meet the burden of showing a case of obviousness relying on a combination of documents the Examiner must show that there would have been motivation to one of ordinary skill in the art to have made the combination. Numerous reported cases have stated this. For example, in In re Fritch, 23 USPQ 2d 1780 (1992), the Federal Circuit stated that the Examiner:

> "can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references."
>
> "Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination."
>
> "It is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious."

In seeking to show the obviousness of the present invention, the Examiner has combined documents in mosaic fashion in a manner not even remotely suggested by the documents themselves. Prior to the making of the present invention there would have been absolutely no motivation for the person skilled in the art to combine the references.

Neither Mullis et al nor Herzog et al is concerned with immunoglobulins at all. Skerra et al provides teaching of almost negligible relevance, if that, to the present invention.

Kabat provides raw data with no indication of any usefulness. None of the documents provides any teaching of relevance to the cloning of immunoglobulin variable domain sequences for expression of a repertoire. There is not even a suggestion of the idea underlying the present invention.

The Examiner states that "one of ordinary skill in the art would have recognized that the general teachings of either reference [Mullis et al; Herzog et al] could have been applied to other systems, e.g., the cloning of Fv-encoding DNAs as disclosed by Skerra et al."

Firstly, there is no evidence that this would have been true.

Secondly, combining the teachings of the documents in the manner suggested by the Examiner does not lead to the present invention.

Mullis and Herzog teach that PCR can be used if there is sequence information available for the *specific* sequence of interest. If only the protein is available, Mullis et al teaches that it should be partially sequenced and the amino acid sequence information used in the design of a pool of oligonucleotides representing all possibilities for the underlying nucleic acid sequence to make sure that the single *specific* sequence of interest is found. Herzog et al teach that more than one primer can be used where there is more than one sequence of interest, provided, and this is consistent with Mullis et al, each primer is an exact match for *each* corresponding sequence sought.

Neither document teaches that it is possible to use primers which each bind more than one different sequence of interest. Furthermore, neither document suggests that primers can be used to clone a repertoire of immunoglobulin variable domains. The suggestion of Mullis et al that their process can be applied to any sequence may be true in a general sense but, in

any specific case, requires the availability of suitable primers. In fact, it requires that it is possible to design suitable primers in that specific case. Amino acid sequencing of a protein can be used to provide information enough to allow design of primers for a specific case. However, there is nothing in any of the citations which suggests what to do if confronted with a very large number of different, inherently variable sequences, i.e. a repertoire. The teaching of Mullis is to sequence each protein in the repertoire and then design a pool of primers for each protein. Such an approach is ridiculous given the numbers involved with immunoglobulins and would not be given serious thought by one skilled in the art.

Kabat does give some information about immunoglobulin sequences. However, this would only have provided a "short cut" for those specific sequences listed in Kabat if the information were combined with the teaching of Mullis, given the fact that Mullis teaches the need for sequence information on each protein for which the encoding sequence is sought. Kabat does not teach or suggest that there is sequence conservation such as to allow primers to be made and used in the cloning of immunoglobulin variable domain gene repertoires.

The alternative combination of Kabat with Herzog does not remedy this, Herzog also teaching that specific primers are needed for each sequence of interest.

Additionally, there is nothing in Skerra et al to provide the modifications of the combined teachings of Mullis et al, or Herzog et al, with Kabat. Skerra is unconcerned with immunoglobulin repertoires.

Further in support of the unobviousness of the present invention, reference is made to the paper by Larrick et al listed in the Information Disclosure Statement signed by the

Examiner, on August 15, 1991, in parent application S.N. 07/580,374. This document illustrates the state of the art prior to the present invention.

In essence, Larrick et al followed the teaching of Mullis et al and designed a back (5') primer for an immunoglobulin sequence by deduction of the nucleic acid sequence from the protein sequence, determined by N-terminal protein microsequencing. For expression of immunoglobulin from B lymphocytes via PCR amplification they envisage a complex procedure involving cloning B lymphocytes, making cDNA, using PCR to obtain variable region sequences, primers having been designed individually in the above manner, and then constructing a CDR-substituted recombinant immunoglobulin which is then expressed.

There is absolutely no suggestion that there is sufficient conservation of nucleotides at the 5' end of variable domain coding sequences to enable a limited number of primers to be used in cloning a repertoire of variable domain sequences. There is no conception of the idea of cloning a repertoire for expression. As discussed, the method taught by Mullis et al, used by Larrick et al, would be wholly inappropriate for the cloning for expression of a repertoire of a large number of sequences, as in the present invention. It is inconceivable that one would sequence every immunoglobulin, design a primer (or a pool taking into account the degeneracy of the genetic code) for each one, then perform individual PCR for each one. The fact that Larrick et al followed the teaching of Mullis et al supports the unobviousness of the present invention, illustrating the fact that there was in the art no suggestion of doing what is presently claimed.

Prior Art Rejection 3

Schoemaker is cited only in the third prior art rejection in which four references are combined (Mullis or Herzog are counted as one reference).

Schoemaker et al

This document discloses "heterochain antibodies" consisting of heavy and light chains form different sources. The authors concerned themselves with taking a heavy chain form one antibody of known specificity and combining it with a light chain from a different antibody of the same specificity to produce a heterochain antibody which might be better than the "parents". In no way does Schoemaker et al remedy the deficiencies of Skerra, Mullis, Herzog and Kabat in rendering obvious the invention of claims 34-37 and 46-56.

9. CONCLUSIONS

The Examiner's art based rejections are in error and must be reversed as not in accord with the proper standards for reviewing patentability under 35 U.S.C. §103. Such action is solicited.

Respectfully submitted,

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APPENDIX

33. A method of cloning sequences (target sequences) each containing a sequence encoding at least part of an immunoglobulin variable domain, which method comprises

providing a sample repertoire of nucleic acid containing target sequences, and

using forward and back primers in the copying and cloning of the target sequences for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of the target sequences.

34. A method according to claim 33 which method comprises:

(a) providing a sample repertoire of double-stranded nucleic acid containing target sequences;

(b) causing the two strands of the double-stranded nucleic acid to be separated;

(c) annealing to the sample a forward and a back oligonucleotide primer, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of the target sequences, under conditions which allow the primers to hybridize specifically to the nucleic acid;

(d) treating the annealed sample with a DNA polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing double-stranded nucleic acid;

(e) repeating steps (b) to (d), thereby producing some double-stranded DNA (product DNA) containing only the target sequences;

(f) cloning product DNA into expression vectors for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain.

35. A method according to claim 34 wherein steps (b) to (d) are repeated a plurality of times before step (f).

36. A method according to claim 33, which comprises:

(a) providing a repertoire of mRNA;

(b) annealing to the mRNA an oligonucleotide primer specific for a sequence at or adjacent the 3' end of each of the target sequences on the sense strands, under conditions which allow the primer to hybridize specifically to the nucleic acid;

(c) treating the primer-annealed mRNA with a polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing antisense cDNA;

(d) annealing to the cDNA an oligonucleotide primer specific for a sequence at or adjacent the 3' end of each of the target sequences on the antisense strands,

under conditions which allow the primer to hybridize specifically to the nucleic acid;

(e) treating the primer-annealed cDNA with a polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing double-stranded DNA (product DNA);

(f) cloning product DNA into expression vectors for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain.

37. A method according to claim 36 wherein, after step (e) the following steps are performed before step (f);

(i) causing the two strands of the product DNA to be separated;

(ii) annealing to the separated strands a forward and a back oligonucleotide primer, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of the target sequences, under conditions which allow the primers to hybridize specifically to the nucleic acid;

(iii) treating the annealed sample with a DNA polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing double-stranded nucleic acid.

38. A method according to claim 33 wherein the back primer is specific for a sequence at or adjacent the 3' end of the antisense strand of the sequences which are contained in the target sequences and which each encode at least part of an immunoglobulin variable domain.

39. A method according to claim 33 wherein the sample repertoire of double-stranded nucleic acid is derived from lymphocytes.

40. A method according to claim 39 wherein the lymphocytes are derived from an animal or human mounting an immune response to an antigen.

41. A method according to claim 39 wherein the lymphocytes are derived from a patient with an autoimmune disease.

42. A method according to claim 33 wherein the sample repertoire of nucleic acid is derived from rearranged immunoglobulin variable region genes.

43. A method according to claim 33 wherein the sample repertoire of nucleic acid is genomic DNA.

44. A method according to claim 33 wherein the sample repertoire of nucleic acid is derived from unrearranged immunoglobulin variable region genes.

45. A method according to claim 33 wherein the target sequence contains a sequence encoding a variable domain from an immunoglobulin heavy chain.

46. A method according to claim 45 wherein the product DNA is inserted into an expression vector for expression of single domain ligands selectable by their binding affinity for antigen.

47. A method according to claim 33 wherein product DNA is inserted into an expression vector for expression of antibodies or antibody fragments selectable by their binding affinity for antigen.

48. A method according to claim 33 wherein the product DNA is inserted into an expression vector for expression alone.

49. The method of claim 33 wherein the product DNA is inserted into an expression vector for expression in combination with a complementary variable domain.

50. A method according to claim 33 wherein the product DNA is inserted into an expression vector already containing sequences encoding one or more constant domains for expression.

51. A method according to claim 33 wherein the product DNA is inserted into an expression vector for expression as fusion proteins.

52. A method according to claim 33 wherein the product DNA is inserted into an expression vector for expression with peptide tags.

53. A method according to claim 33 wherein product DNA containing sequences encoding at least immunoglobulin heavy chain variable domains is inserted into expression vectors along with product DNA containing sequences encoding at least immunoglobulin light chain variable domains for expression of a combinatorial repertoire of complementary variable domains.

54. A method according to claim 53 wherein the product DNA is inserted into an expression vector already containing sequences encoding one or more constant domains for expression.

55. A method according to claim 53 wherein product DNA is inserted into expression vectors for expression as fusion proteins.

56. A method according to claim 53 wherein the product DNA is inserted into an expression vector for expression with peptide tags.

57. A method according to claim 33 wherein the forward and back primers are provided as single oligonucleotides.

58. A method according to claim 33 wherein the forward primers are supplied as a mixture of oligonucleotides.

59. A method according to claim 33 wherein the back primers are supplied as a mixture of oligonucleotides.

60. A method according to claim 33 wherein each primer includes a sequence encoding a restriction enzyme recognition site.

61. A method according to claim 60 wherein the restriction enzyme recognition site is located in the sequence which is annealed to the nucleic acid.

62. A method according to claim 33 wherein the back primer is a general primer useful for cloning a desired antibody specificity from a specific species.

63. A method according to claim 33 wherein the back primer is a mixture of primers having a variety of sequences designed to be complementary to the various families of VH, Vk or Vλ sequences.